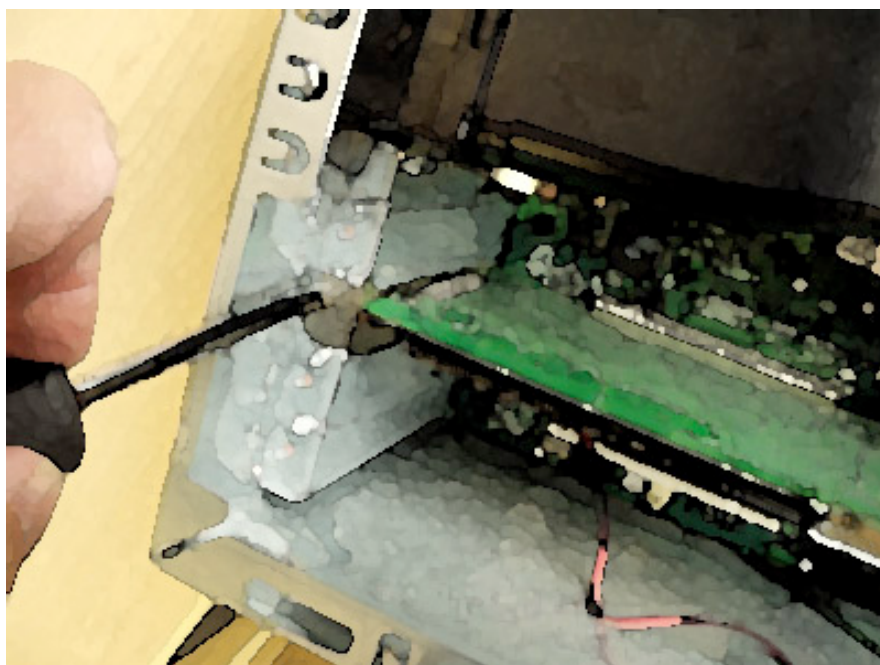


# OPTICON INSTALLATION GUIDE



Version 4.3

Bio-Rad Technical Support  
Dave Palmer – 11 September 2007

# PURPOSE

This guide provides a step-by-step procedure for the re-installation of a single-color Opticon or two-color Opticon2 real-time PCR system\*.

In cases where the hard drive of an original Opticon's computer has crashed, this information will assist the user in configuring the new computer to work with the existing Opticon. In addition, this guide is also useful for anyone wishing to upgrade to the latest version of the Opticon Monitor software (currently version 3.1), from any previous version.



**NOTE:** This guide is NOT recommended for brand-new Opticon installations. If you are installing a newly-purchased system, please follow the directions that came with the system itself, these are also **reprinted in Appendix E** of this guide.



**NOTE:** Opticon installation is a multi-step procedure, involving the installation of numerous drivers, software packages, and hardware connections. **Please follow the directions in this guide carefully and precisely.** At each stage of this procedure, if anything unusual or unexpected occurs, please contact Bio-Rad Technical Support (800-424-6723 Option 2 Option 1) before proceeding further. Tech support can assist over the phone at any stage of the installation process.

Thank you,  
Bio-Rad Technical Support

*\* Because the installation processes for the Opticon and the Opticon2 are slightly different, this guide will use the terminology "Opticon1" to refer to the single-color Opticon; "Opticon2" to refer to the two-color Opticon2 system; and also "Opticon" to refer in generic terms to either of the two instruments.*

# Quick Guide

## Step 1: Preliminary Computer Preparation

- 1) Back up the old \opticon monitor\ folders
- 2) Upgrade to Win XP Pro
- 3) Set COM port to COM2

## Step 2: Install the NIDAQ Card

- 1) Download the software and prepare the computer
  - a) Download software (Nidaq692.EXE)
  - b) Power off PC.
  - c) Remove NIDAQ card.
  - d) Power on PC.
- 2) Begin the driver installation and reinstall the NIDAQ card.
  - a) Move Nidaq692.EXE software file to temporary directory.
  - b) Run setup.exe
  - c) Follow default prompts to install driver.
  - d) Power off PC.
  - e) Re-install NIDAQ card.
- 3) Activate NIDAQ
  - a) Run Measurements and Automation Software
  - b) F5-Refresh to capture instruments
  - c) Find NIDAQ card in instrument tree on left
  - d) Right-click and run Test Panel
  - e) Exit out of M&A software.

## Step 3: Install the Opticon Monitor Software

- 1) Install Opticon Monitor 3.1
  - a) Download OM 3.1 from our website:
  - b) Extract the .zip archive and copy the OM3\_1.exe file to the desktop.
  - c) Copy the two files in the DNA Engine Firmware folder to the desktop:
  - d) Double-click on the OM3\_1 file to install the Opticon Monitor software.

## Step 4: Upgrade the Cyclor Firmware

- 1) Connect opticon to PC via NIDAQ and serial cables.
- 2) Run firmware upgrade on PTC-200.
  - a) Double-click cyclor update utility, now on desktop, to extract.
  - b) Doubleclick 33GEngine firmware to extract. Follow prompts.
  - c) Run cyclor update utility (a new icon is on the desktop).
  - d) Select COM2 under Communications menu.
  - e) Verify engine firmware available to upgrade in left box is 3.3G
  - f) Click radio button above DNA Engine (left of the three)
  - g) Click continue
  - h) Ensure cables are plugged in, follow prompts. The upgrade takes 20 minutes or so.
- 3) After firmware update is complete, BE SURE TO POWER CYCLE OPTICON.
  - a) Turn the Opticon off.
  - b) Turn the Opticon back on.

## Step 5: Verify that Cyclor and NIDAQ are working.

- 1) Run Enumerator and verify both cyclor and NIDAQ are seen.
  - a) Enumerator is a program called IEnum3.exe in the \opticon monitor 3\utils\ directory.
  - b) Scan for all devices.
  - c) Verify the NIDAQ card has been found.
  - d) Verify that the thermal cyclor is found, and is on COM2
  - e) Verify that cyclor firmware is 3.3G (double-click on PTC-200, give command \*idn? to unit)
  - f) Close the enumerator.

## Step 6: Import Previous Instrument Settings.

- 1) If no calibration files are available, skip immediately to Appendix A or B.
- 2) If you have the calibration files and are installing an Opticon 1.
  - a) Locate your original calibration files. They are located in the path c:\program files\opticon monitor\.
  - b) Copy the files called gain.idx and norms.elh to a flash drive, etc.
  - c) Install the files to the following location on your new computer:
  - d) Start Opticon Monitor and select the instrument "Opticon". The instrument should be working normally now
- 3) If you have the calibration files and are installing an Opticon 2
  - a) Run OM3.1 software.
  - b) Import previous instrument settings (if you have a backup of your old OM directory)
  - c) Import previous instrument settings (if your files came from CD or email)
  - d) Restart system.
  - e) Select Opticon2 as the instrument. Verify that instrument is ready.
  - f) You're done!

Appendix A: Recalibrating your Opticon 1.

Appendix B: Recalibrating your Opticon 2.

Appendix C: Testing the computer serial port and cable.

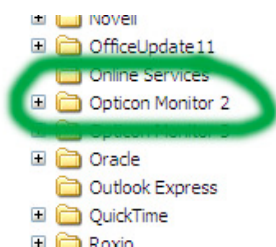
Appendix D: General troubleshooting tips.

Appendix E: New Instrument Installation.

# Step 1: Preliminary Computer Preparation

1) **Back up the old \opticon monitor\ folders on the HD of the PC connected to the Opticon.**

*This step is important because you'll need the calibration files to initialize the new installation. You will not be able to connect to the Opticon without these files.*



2) **Upgrade the computer operating system to Win XP Pro (Opticon2 only)**

*It is important to have the correct operating system for your software package. This step is not necessary if you are already running Windows XP Professional.*

**Opticon 2:** you are reinstalling an Opticon2 system, we strongly recommend upgrading to Opticon Monitor 3 at this time. In order to run the OM3 software, you absolutely must be running Windows XP Professional. [Windows Vista is unsupported at this time; do not attempt installation with Vista.]



**Opticon 1:** If you are reinstalling an Opticon1 system, and were previously running the Opticon Monitor version 1.x software, we recommend reinstalling the original software rather than upgrading to Opticon Monitor 3. Only if problems are encountered with a reinstallation of OM1.x (instrument not found errors) do we suggest then upgrading to Opticon Monitor 3 following the path described for an Opticon 2, here and below.

3) **Turn off all of the power save options on your computer**

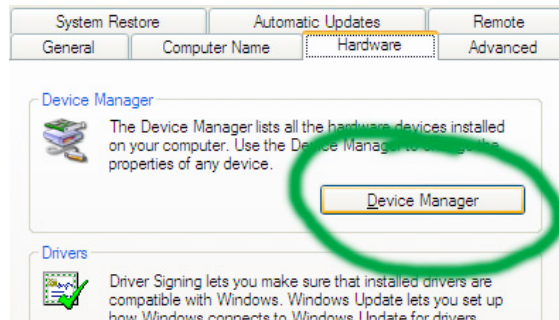
*It is very important to disable all sleep and hibernate modes on your computer. This prevents real-time PCR runs from being prematurely terminated.*

- ✓ Visit Start > Settings > Control Panel > Power Options.
- ✓ Ensure all power save options are set to "Never".

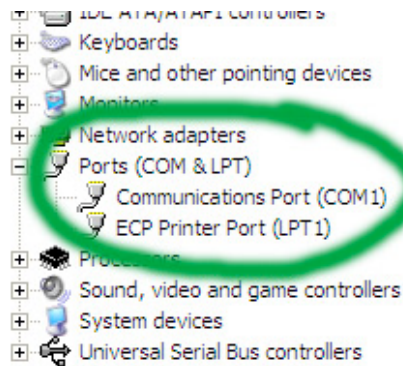
4) **Set COM port settings on serial port to COM2**

*This step is important because OM3.1 requires the Opticon to be located on COM2.*

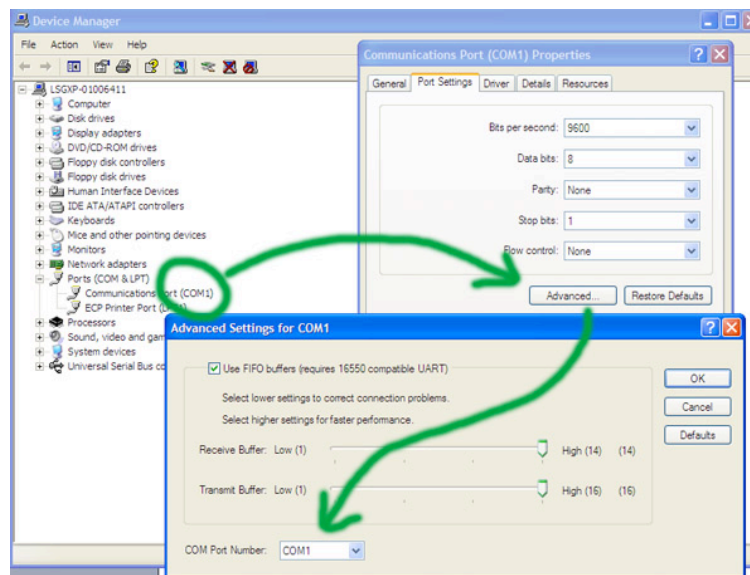
a) Start > Settings > Control Panel > System > Hardware > Device Manager



b) Open Ports tree, right-click on Communications Port (COM1)



c) Choose Properties > Port Settings > Advanced



d) Change com port to COM2.

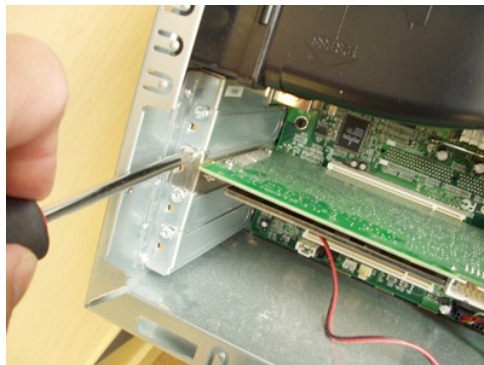
## Step 2: Installing the NIDAQ Card

### 1) Download the software and prepare the computer

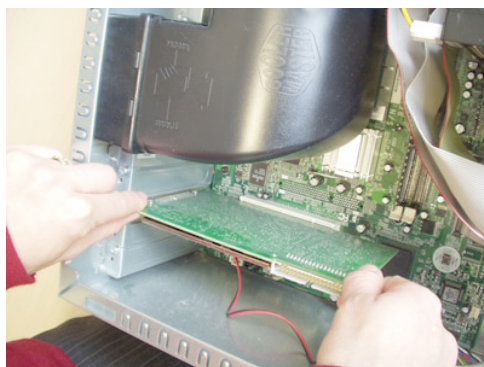
*These steps install the communications card.*

*It is best to install the drivers with the NIDAQ card OUTSIDE of the computer, so the first step involves removing the card.*

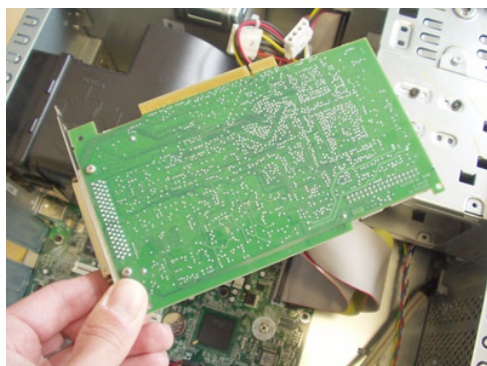
- a) Download software (Nidaq692.EXE) from the National Instruments website:
  - a. Version 6.9.2 (Nidaq692.EXE) is the preferred driver, but is frequently not available on the website. Driver might still be available at <http://joule.ni.com/nidu/cds/view/p/id/824/lang/en>
  - b. A useful alternative is version 7.4.4. Any other driver is **not** recommended.
  - c. Go to [www.ni.com/support](http://www.ni.com/support)
  - d. Choose Support
  - e. Choose Drivers and Updates (left panel)
  - f. Choose Product --> Data Acquisition
  - g. Choose Multifunction DAQ
  - h. Choose Traditional NI-DAQ, version 7.4.4, from list at bottom. This will lead to a 288-megabyte download.
- b) Power off PC. (if NIDAQ card isn't installed yet, skip to the driver installation step)
- c) Remove NIDAQ card.



Unscrew the screw holding the bracket.



Being careful to avoid static discharge (wear wrist strap or touch chassis to ground yourself), carefully grab the corners of the NIDAQ card and pull straight out.

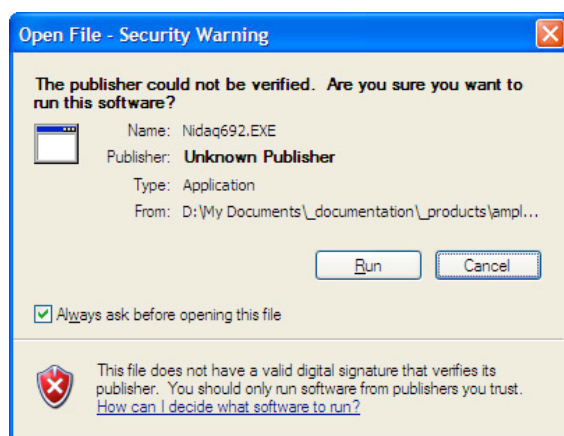


Once the NIDAQ card is removed, carefully set it aside.

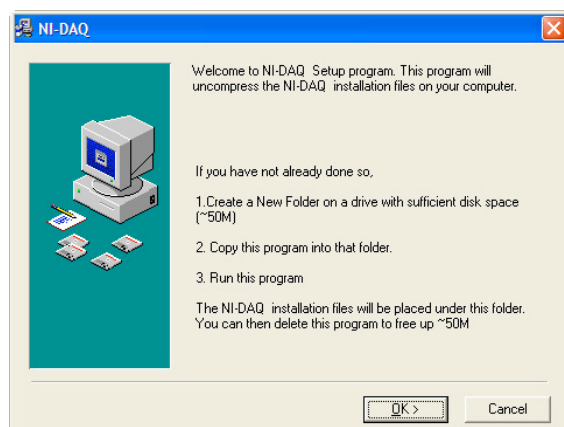
d) Power on PC.

## 2) Begin the driver installation and reinstall the NIDAQ card.

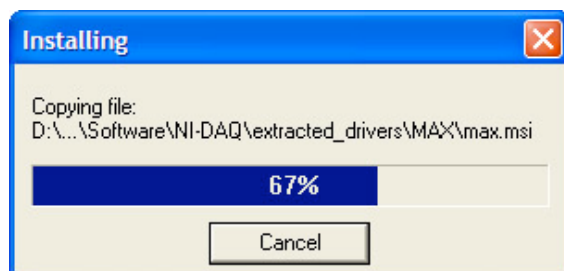
a) Move Nidaq692.EXE software file to temporary directory. Double-click to extract.



A windows security warning is normal.



Follow the prompts.



Files will extract.

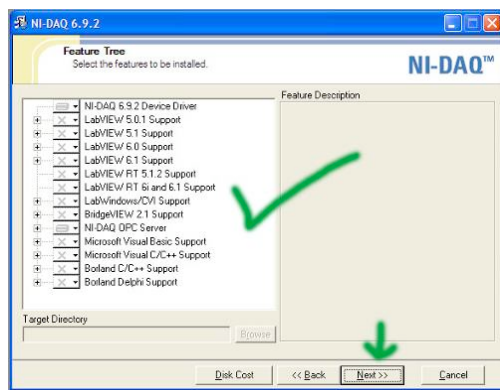
Name	Size	Type	Date Modified
Nidaq692.EXE	63,765 KB	Application	3/20/2006 11:54 AM
LicenseAgreement		File Folder	3/22/2006 4:00 PM
MAX		File Folder	3/22/2006 4:00 PM
nidaq		File Folder	3/22/2006 4:00 PM
PAL		File Folder	3/22/2006 4:00 PM
suppfiles		File Folder	3/22/2006 4:00 PM
autorun.inf	1 KB	Setup Information	1/28/2002 3:11 PM
readme.htm	127 KB	HTML Document	1/28/2002 3:05 PM
readmeD.htm	128 KB	HTML Document	1/28/2002 3:05 PM
readmeF.htm	127 KB	HTML Document	1/28/2002 3:05 PM
readmeJ.htm	299 KB	HTML Document	1/28/2002 3:05 PM
setup.exe	660 KB	Application	1/28/2002 3:11 PM
setup.ini	7 KB	Configuration Settings	7/25/2002 3:20 PM
transforms		File Folder	3/22/2006 4:01 PM

b) Run setup.exe, it is one of the files in the temporary directory you created above.



The NIDAQ card will install.

c) Follow default prompts to install driver.



Accept the feature tree as suggested.

d) Power off PC.

e) Re-install NIDAQ card.

Installation is the reverse of removal.

x) Alternatively:

- i) Download NIDAQ software, move to temporary folder, extract.
- ii) Run setup.exe, follow default prompts.
- iii) Power-cycle PC.
- iv) When Windows Plug-N-Play detects device, point to your temporary folder so it can find drivers.

### 3) Activate NIDAQ

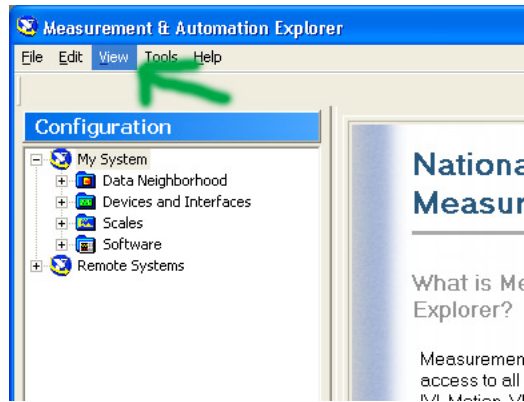
*The communications card will **not** work unless you first run the M&A software.*

a) Run Measurements and Automation Software

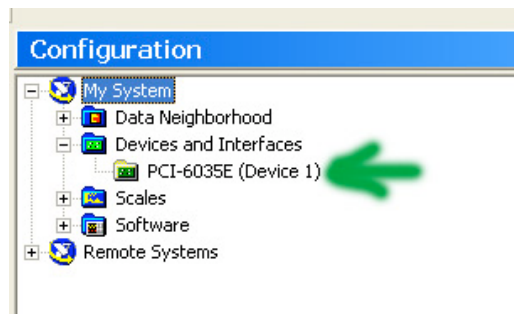


A new icon will have been added to the desktop

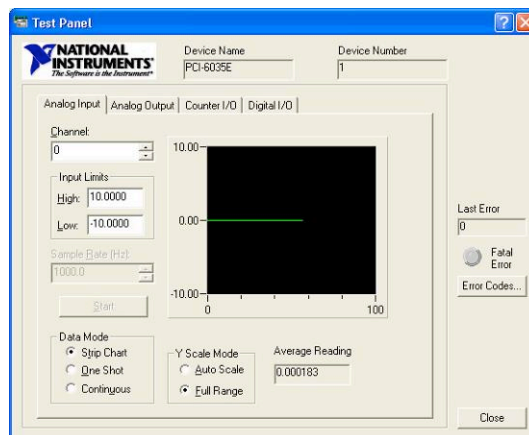
b) F5-Refresh to capture instruments



c) Find NIDAQ card in instrument tree on left



d) Right-click and run Test Panel



If the card is working, you will see a green trace running across screen.

e) Exit out of M&A software.

## Step 3: Installing the Opticon Monitor Software

### 0) Select the software to install

**Opticon 2:** you are reinstalling an Opticon2 system, we strongly recommend upgrading to Opticon Monitor 3 at this time.

**Opticon 1:** If you are reinstalling an Opticon1 system, and were previously running the Opticon Monitor version 1.x software, we recommend reinstalling the original software rather than upgrading to Opticon Monitor 3. Proceed directly to Step 6, Import Previous Instrument Settings. Only if problems are encountered with a reinstallation of OM1.x do we suggest upgrading to Opticon Monitor 3.

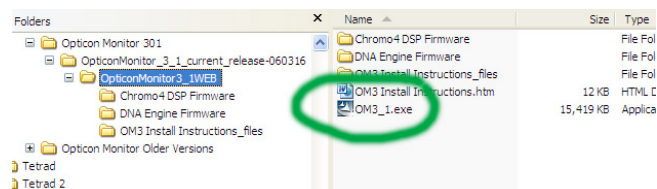
### 1) Install Opticon Monitor 3.1

*This step installs the Opticon Software and utilities.*

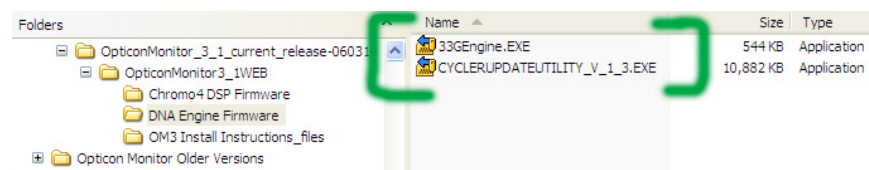
a) Download OM 3.1 from our website:

[http://www.bio-rad.com/LifeScience/jobs/2005/05-0552/OpticonMonitor\\_3\\_1.zip](http://www.bio-rad.com/LifeScience/jobs/2005/05-0552/OpticonMonitor_3_1.zip)

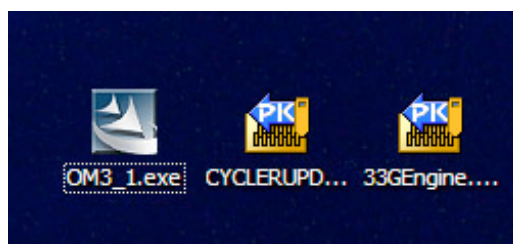
b) Extract the .zip archive and copy the OM3\_1.exe file to the desktop.



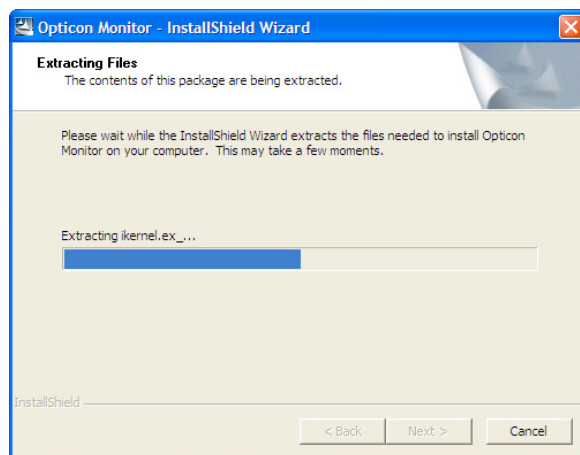
c) Copy the two files in the DNA Engine Firmware folder to the desktop:



You should now have three icons on your desktop:



d) Double-click on the OM3\_1 file to install the Opticon Monitor software.



A dialog box will come up ... follow the prompts.

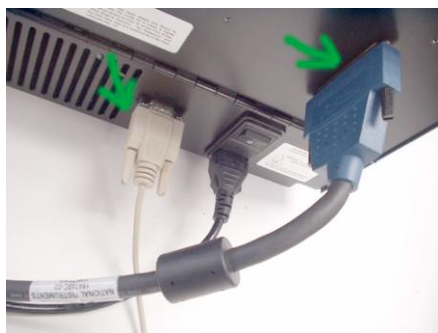
Once the dialog boxes and prompts are finished, the Opticon Monitor will have been installed.

## Step 4: Upgrade the Cyclor Firmware

### 0) Check whether you need to upgrade the firmware.

You **must** upgrade the firmware if you are upgrading to Opticon Monitor 3. If you are reinstalling an Opticon1 with the original Opticon Monitor version 1.x software, then it is not necessary to upgrade the cyclor firmware. If you are installing a NEW instrument, you should be following the directions in Appendix E, and you do not have to upgrade your firmware.

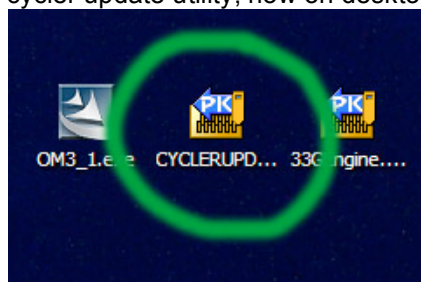
### 1) Connect opticon to PC via NIDAQ and serial cables.



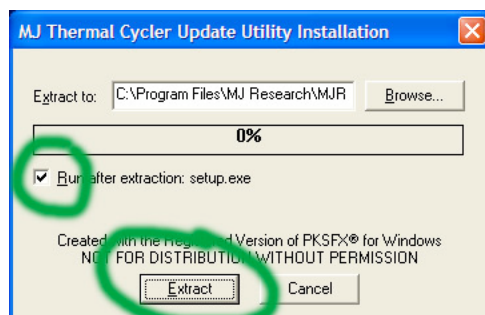
### 2) Run firmware upgrade on PTC-200.

*Opticon Monitor 3.1 absolutely requires the thermal cyclor inside the opticon to have 3.3G firmware.*

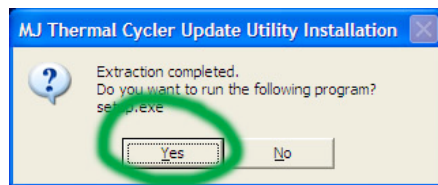
a) Double-click cyclor update utility, now on desktop, to extract.



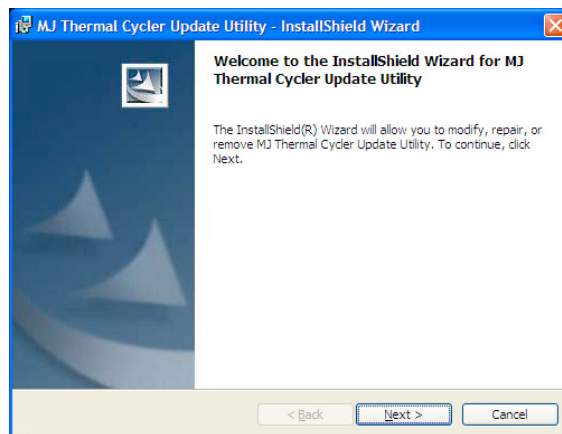
Choose Run after Extraction:



After extraction, let the setup program run:



The installer will then launch. Follow prompts:



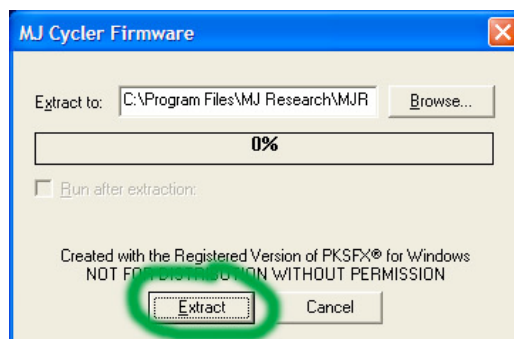
After installation, you will have a new icon on the desktop:



b) Doubleclick 33GEngine firmware to extract. Follow prompts.



Extract the file:

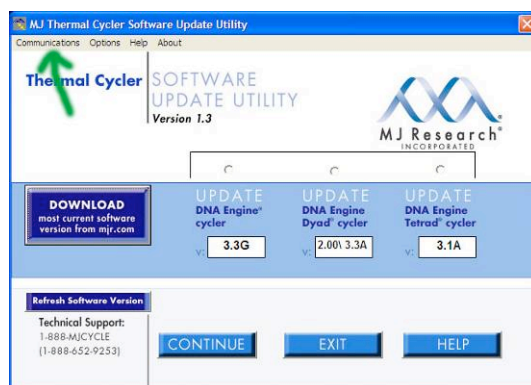


Extraction will complete with no further action required.

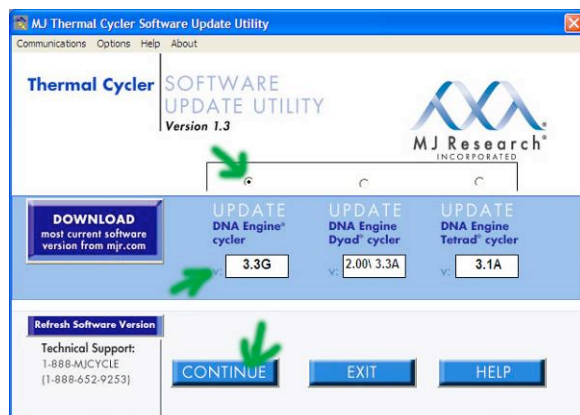
- c) Run cyclor update utility (a new icon is on the desktop). You'll need the Opticon powered on.



- d) Select COM2 under Communications menu.



- e) Verify engine firmware available to upgrade in left box is 3.3G  
f) Click radio button above DNA Engine (left of the three)  
g) Click continue

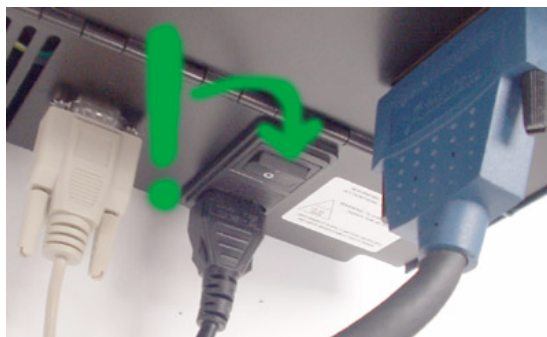


h) Ensure cables are plugged in, follow prompts. The upgrade takes 20 minutes or so.

3) **After firmware update is complete, BE SURE TO POWER CYCLE OPTICON.**

*Power cycling the thermal cycler is **essential**, otherwise firmware will become corrupted.*

- a) Turn the Opticon off.
- b) Turn the Opticon back on.



- c) If a dialog box comes up that says that default programs cannot be erased, click through. This is normal. There is no need to delete these protocols. Since the thermal cycler keypad is inside the opticon and is inaccessible, there is actually no way to, nor any need to, delete these protocols.

## Step 5: Verify that Cyclor and NIDAQ are working.

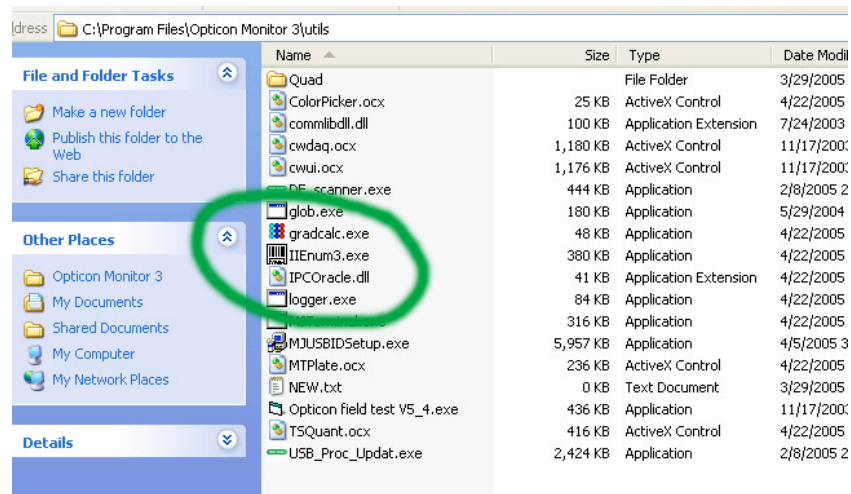
0) These checks are only available with Opticon Monitor version 3.

If you are reinstalling an Opticon1 with the original Opticon Monitor version 1 software, proceed directly to step 6. Otherwise proceed through the following checks.

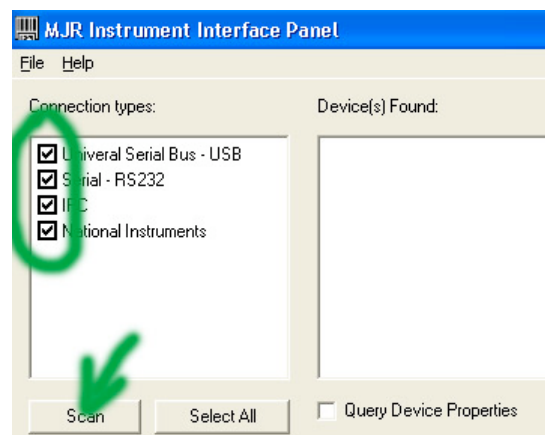
1) Run Enumerator and verify both cyclor and NIDAQ are seen.

*This step verifies everything is okay so far.*

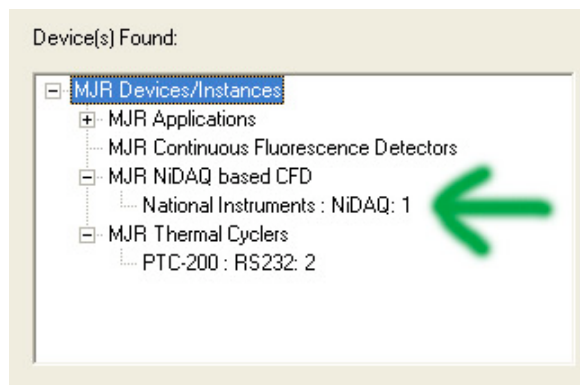
a) Enumerator is a program called IEnum3.exe in the \opticon monitor 3\utils\ directory.



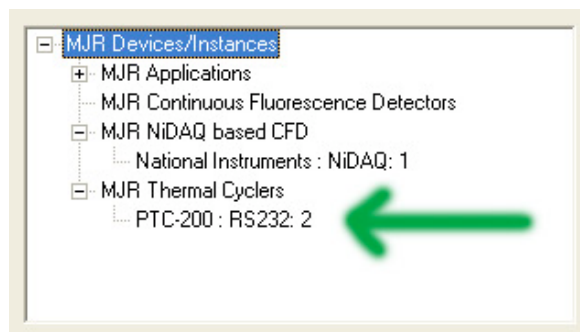
b) Scan for all devices.



c) Verify the NIDAQ card has been found.



d) Verify that the thermal cyclers are found, and are on COM2



The enumerator should report PTC-200: RS232: 2. The last numerical digit in the ID listing is the port number, it must be 2. *(If this line does **not** show up, troubleshoot the computer serial port and cable using the steps in Appendix C).*

e) Verify that cycler firmware is 3.3G (double-click on PTC-200, give command \*idn? to unit)

```

C:\Program Files\Opticon Monitor 3\utils\MJTerminal.exe

command:*idn?
Send(SUCCEEDED): "*idn?"
Listening...
RCU 30:MJ RESEARCH,PTC-200,20802,3.3G<0>
  
```

The firmware version is listed right after the serial number.

f) Close the enumerator.

Once NIDAQ presence has been confirmed, and PTC-200 is present and has 3.3G, close the enumerator.

## Step 6: Import Previous Instrument Settings.

### 1) If no calibration files are available, skip immediately to Appendix A or B.

*You can only import previous instrument settings if they actually exist. If you are recovering from a hard drive failure or a lost computer, and you have never made a backup of your previous system, and you have lost your dye files, then you will not be able to import previous settings. You will have to recalibrate as instructed in Appendix A if you have an Opticon1 or Appendix B if you have an Opticon2.*

### 2) If you have the calibration files and are installing an Opticon 1.

*The Opticon1 is a single-color instrument and uses a very simple calibration system consisting of only two files, gain.idx and norms.elh. These files need to be moved to the correct locations in the new software directories.*

- a) Locate your original calibration files. They are located in the path c:\program files\opticon monitor\.
- b) Copy the files called gain.idx and norms.elh to a flash drive, etc.
- c) Install the files to the following location on your new computer:  
Opticon Monitor version 1.x: c:\program files\opticon monitor\  
Opticon Monitor version 3.1: c:\program files\opticon monitor 3\instrument\opticon\
- d) Start Opticon Monitor and select the instrument "Opticon". The instrument should be working normally now

### 3) If you have the calibration files and are installing an Opticon 2.

*Congratulations on having kept backups of your important calibration files. Proceed with the following steps.*

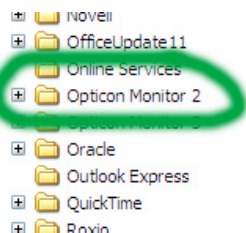
#### a) Run OM3.1 software.

*It is normal for the OM software to say "No Instrument" at this point. The settings from the previous installation MUST be imported in order for OM3.1 to be able to "see" the opticon, even though it is physically connected.*

#### b) Import previous instrument settings (if you have a backup of your old OM directory)

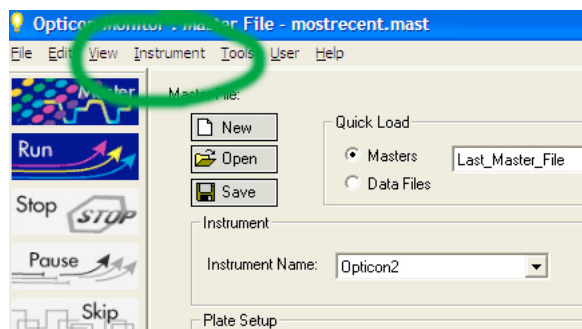
*This step collects the dye files from the older installation, and enables the OM3.1 to talk to the Opticon. If you are importing calibration files from a CD or from email, proceed to the next step.*

- i) Copy the old Opticon Monitor folder to a temporary directory on your C: drive.



- ii) Change instrument to "Settings for Opticon2"

- iii) Under Instrument tab, click "import previous instrument settings"...  
...and browse for the older folder, then click Search.



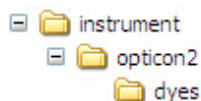
- iv) Once older folder is identified, it will show in the window.
- v) Select instrument and choose "import".

#### c) Import previous instrument settings (if your files came from CD or email)



**TIP:** Bio-Rad technical support has factory calibration files for a limited number of instruments. You may contact us at 800-424-6723 to see if we have your files available to send to you. If we do not have your files, and you have lost your CD containing the calibration files, you will have to recalibrate as directed in Appendix B.

- i) Create a folder called "instrument" on desktop.
- iii) Create a subfolder called "opticon2" within the instrument folder.
- iv) Create a subfolder called "dyes" within the "opticon2" folder.



The directory tree should look exactly as shown above.

- v) Move all files except new\_machopts and/or machopts into \dyes\
- vi) Move new\_machopts and/or machopts into \opticon2\.
- vii) Files may now be imported using *Instrument* then *Import Previous Instrument Settings* using the main menu in Opticon Monitor 3. Browse for the *instrument* directory that you have created on the desktop and import the files.

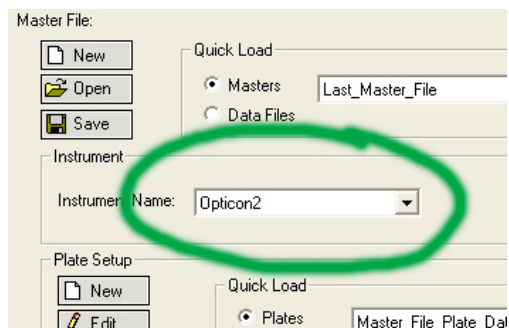
TIP: Spelling, capitalization, spacing, etc is CRITICAL with above filenames.

TIP: This import method is an effective way to load just about any Opticon2 calibration data set, from almost any installation or source.

#### d) Restart system.

- i) Close OM software
- ii) Power down Opticon, power down PC, restart PC, restart Opticon.
- iii) Reopen OM software

#### e) Select Opticon2 as the instrument. Verify that instrument is ready.



Be sure the correct instrument is selected.



If all went well, you'll see the familiar "Ready" status.

f) **You're done!**

# Appendix A: Recalibrating your Opticon1.

## 1) Only recalibrate if you have entirely lost your original calibration files.

*The Opticon is calibrated at the factory, and there is no need to recalibrate after a reinstallation. The only time in which you should need to proceed through this section would be if you had entirely lost your original gain.idx and norms.elh files.*



**TIP:** Bio-Rad technical support has factory calibration files for a limited number of instruments. Before beginning your calibration procedure, it may be worthwhile to contact us at 800-424-6723 to see if we have your files available to send to you. If it turns out that we can send you your files, you can then skip recalibration and proceed through Step 6.

## 2) Install Opticon Monitor version 1

*The following methods will ONLY work for Opticon Monitor version 1, ie. OM 1.08.*

## 3) Determine your instruments photomultiplier tube gain settings.

*Since all of your previous instrument settings are lost, you will have to use the advanced field test software to discover the gain settings from the photomultiplier tubes within the Opticon. Once you know the values, you can edit the gain.idx file to reflect the proper settings for your Opticon.*

- a) Browse to the following directory:

C:\program files\Opticon Monitor\utils\

- b) Double-click on the program **Opticon field test V5\_4.exe** (or similar)  
c) Click on **Start Full Test**. Follow prompts.

*You need to run the full test so that the software knows you are running an Opticon 1 system. Follow the instructions on the screen. Ignore any errors that are reported by choosing [CANCEL]. Once the test has completed, the System Type will read Opticon 1.*

- d) Click on **Advanced Test** and locate the PMT gain settings.

*On the left hand side of the Advanced Test Utility is the PMT panel. There are two PMT gain control sliders, one above the other. The gain values are expressed as a three-digit number followed by the word "ON", for example **946 ON**. Record the three-digit value over the top slider.*

## 4) Enter the gain settings into the gain.idx file.

- a) Open or create a file called gain.idx in the Opticon Monitor directory using Windows Notepad.

The file should consist of four, and only four, characters. Specifically, a decimal point followed by the three digits representing the photomultiplier gain value from the test above. For example:

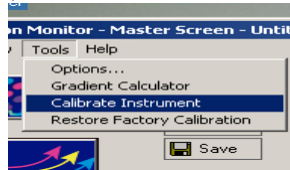
**.946**

Absolutely no other characters, line feeds, or spaces should be included in this file.

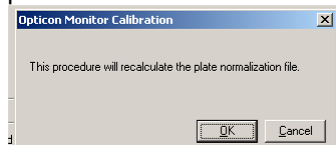
- b) Close the opts file, re-open it, and ensure that your changes have been saved.

**5) Perform the instrument calibration steps.**

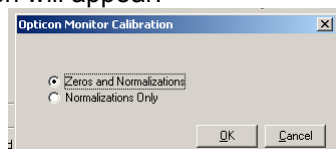
- a) You will need to have the following items available:
- a. Empty black plate
    - i. This is simply a black plate (ie. HSP9666) with no sealers attached.
  - b. Sealed white plate containing 600nM fluorescein.
    - i. Start with a white plate (ie. HSP9665)
    - ii. Prepare 10ml of fluorescein dye solution. Suggest 170-8780 (1mM fluorescein dye stock) diluted 1:1600. Do this by adding 6ul of the dye stock in 10ml water.
    - iii. Seal the plate. Suggest optical flat caps (ie. TCS0803), may use film if more convenient.
- b) Start Opticon Monitor.
- c) From the TOOLS tab, select CALIBRATE INSTRUMENT.



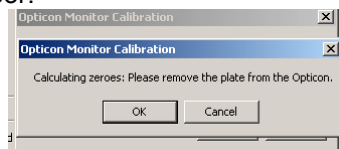
- d) You will see a pop-up screen:



- e) At this time, type the word **z e r o** . This activates a hidden menu.
- f) A new pop-up screen will appear.

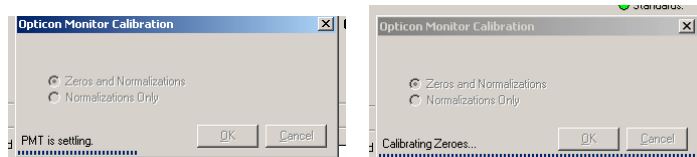


- g) Select ZEROS AND NORMALIZATIONS.
- h) Press OK.
- i) At the screen prompt that tells you to remove the plate, instead insert the EMPTY BLACK PLATE and close door.

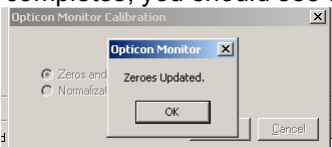


- j) Press OK.

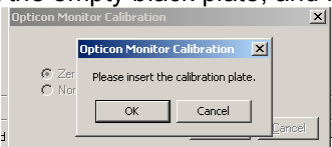
- k) The calibration procedure will then run.



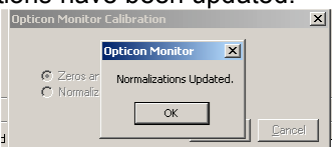
- l) Once the calibration completes, you should see a prompt that zeroes have been updated.



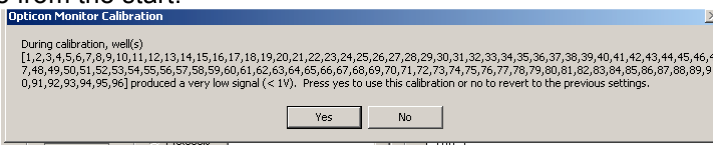
- m) Press OK. Take out the empty black plate, and insert the calibration plate.



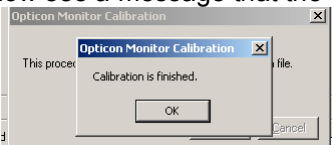
- n) Press OK. The calibration will then run with screens similar to those above, and will report back that Normalizations have been updated.



If you instead see a screen that indicates certain wells had very low signal, go back and check your calculations, dilutions, and pipetting for the SYBR plate. Also check the plate for physical defects, missing wells, bubbles, etc. Click "No", correct your plate, and repeat the calibration procedure from the start.



- o) Press OK. You will now see a message that the calibrations have been updated.



- p) Press OK. The procedure is finished. Your instrument is now ready to use.

- q) Back up your c:\program files\opticon monitor\ directory so that you do not lose your dye files again.

# Appendix B: Recalibrating your Opticon 2.

## 1) Only recalibrate if you have entirely lost your original dye files.

*The Opticon is calibrated at the factory, and there is no need to recalibrate after a reinstallation. The only time in which you should need to proceed through this section would be if you had entirely lost all of your original dye calibration files.*



**TIP:** Bio-Rad technical support has factory calibration files for a limited number of instruments. Before beginning your calibration procedure, it may be worthwhile to contact us at 800-424-6723 to see if we have your files available to send to you. If it turns out that we can send you your files, you can then skip recalibration and proceed through Step 6.

## 2) Download, unzip, and install the unit-0 file.

*In order to allow the computer to communicate with the Opticon 2, you will need to create a new blank set of dye files and communications instructions.*

a) You can download the unit-0.zip file from the following location.

<http://zdap.com/94547/unit-0.zip>

b) Unzip the unit-0.zip file, and move the components to create the following directory structure:

```
C:\program files\Opticon Monitor 3\instrument\opticon2\unit-0\opts
C:\program files\Opticon Monitor 3\instrument\opticon2\unit-0\dyes\id1_zero
C:\program files\Opticon Monitor 3\instrument\opticon2\unit-0\dyes\id2_zero
```

d) Double-click on the opts file, open with WordPad, and confirm that it has the following line:

```
cycler.conn:com2
```

## 3) Use the Advanced Field Test utility to determine the PMT gain settings.

*Since all of your previous instrument settings are lost, you will have to use the advanced field test software to discover the gain settings from the photomultiplier tubes within the Opticon. Once you know the values, you can edit the opts file to reflect the proper settings for your Opticon.*

r) Browse to the following directory:

```
C:\program files\Opticon Monitor 3\utils\
```

s) Double-click on the program **Opticon field test V5\_4.exe**

t) Click on **Start Full Test**. Follow prompts.

*You need to run the full test so that the software knows you are running an Opticon 2 system. Follow the instructions on the screen. Ignore any errors that are reported by choosing [CANCEL]. Once the test has completed, the System Type will read Opticon 2.*

u) Click on **Advanced Test** and locate the PMT gain settings.

*On the left hand side of the Advanced Test Utility is the PMT panel. There are two PMT*

gain control sliders, one above the other. The gain values are expressed as a three-digit number followed by the word "ON", for example **946 ON** or **644 ON**, and they are located at the top of each slider. Record these values.

- v) Replace the gain settings in your current opts file with the new settings.

gain0:0.808000      ← Replace the "808" with the value for PMT1  
gain1:0.690000      ← Replace the "690" with the value for PMT2

- w) Close the opts file, re-open it, and ensure that your changes have been saved.

#### 4) Open the Opticon Monitor software and calibrate the instrument.

*Since all of the calibration dye files have been lost, recalibration is essential.*

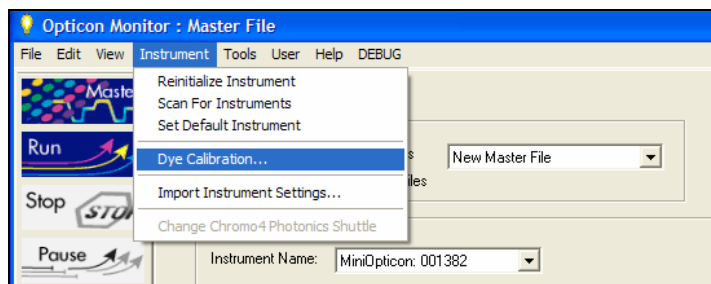
- a) Ensure you have your calibration dyes, empty plates, and flat cap seals ready.

*You will need an empty clear plate (MLL-9601) and an empty white plate (MLL-9651). You will also need optical flat caps (TCS-0803). Common calibration dyes may be obtained directly from Bio-Rad. The dyes come in 5 x 1ml aliquots, and have the following part numbers:*

10006045, SYBR Green I  
10006046, FAM  
10006047, TET  
10006048, HEX

*If you need to calibrate with a different dye, you will have to have it prepared separately. A dye concentration of 0.6  $\mu$ M is a good starting point.*

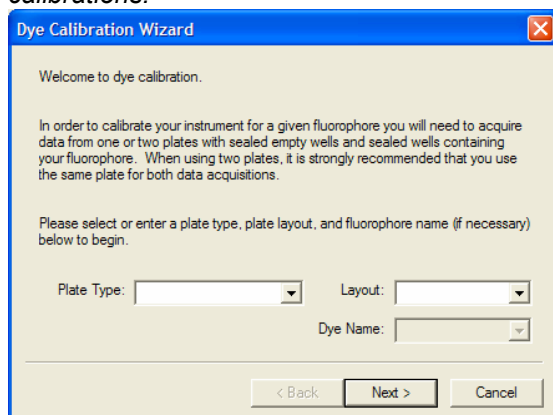
- b) Open the Opticon Monitor software and select Opticon 2 from the Instrument Name panel.  
c) Select **Dye Calibration** from the **Instrument** menu.



- d) The Dye Calibration Wizard will launch and will provide instructions for calibrating.

*Dye calibrations are performed by first scanning a blank plate (an empty microplate that is sealed with clear optical caps), followed by scanning the same plate containing the fluorophore of interest (also sealed with clear optical caps). It is strongly recommended the same plate be used for both the "empty" plate read and the "dye" plate read,*

*variations from plate to plate can be significant and interfere with obtaining optimal calibrations.*



The screenshot shows the 'Dye Calibration Wizard' window. It has a blue title bar with the text 'Dye Calibration Wizard' and a close button. The main area is white with a blue border. It contains the following text: 'Welcome to dye calibration.' followed by a paragraph: 'In order to calibrate your instrument for a given fluorophore you will need to acquire data from one or two plates with sealed empty wells and sealed wells containing your fluorophore. When using two plates, it is strongly recommended that you use the same plate for both data acquisitions.' Below this is another paragraph: 'Please select or enter a plate type, plate layout, and fluorophore name (if necessary) below to begin.' There are three dropdown menus: 'Plate Type:', 'Layout:', and 'Dye Name:'. At the bottom are three buttons: '< Back', 'Next >', and 'Cancel'.

*Follow the instructions provided by the Wizard and begin by selecting or entering a Plate type into the appropriate field on the first screen. This plate type description will identify the type of PCR plate used for this particular calibration (e.g., MJ White, MJ Clear).*

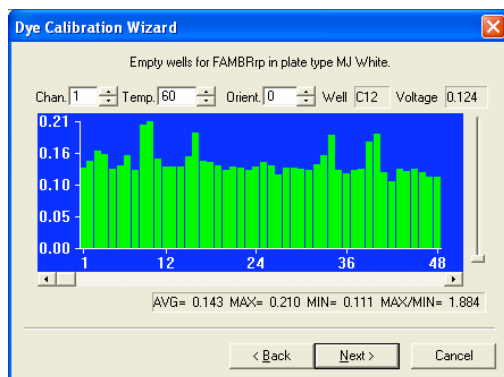
*After selecting a plate type, select FULL from the Layout drop-down list. This is the only Layout option you should select when running a calibration.*

*Next, enter the dye to be calibrated or select the dye from the Dye name drop-down list (e.g., FAM, SBG1, HEX, or TET).*

*Proceed to the next screen by clicking on the Next button.*

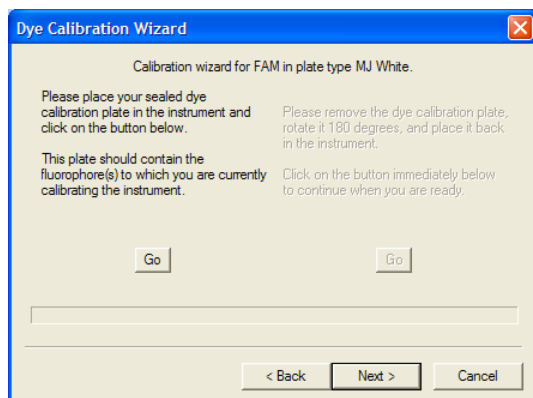
*Insert the sealed **empty** plate, close the door and click on the Go button. The Opticon system will begin the calibration procedure.*

*After the empty plate has been scanned, you will be able to view the acquired data and check it for anomalies that may indicate a problem with the plate (e.g., unusually high signal in certain wells). Variability up to 2- to 3-fold is acceptable.*



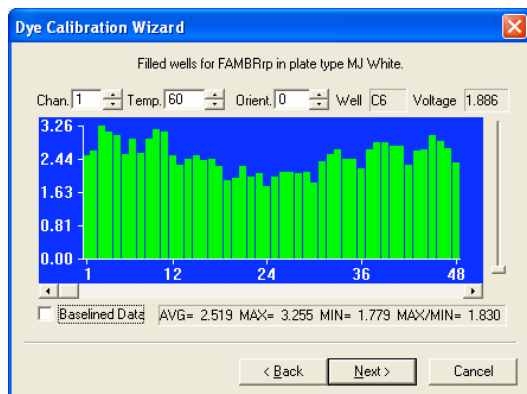
*Click on the Next button to proceed to the dye plate section.*

*Remove the empty plate from the instrument. Remove the optical caps and fill each well with 50µl of calibration dye..*



*Seal the filled dye plate with clear optical caps and place the plate in the instrument. Close the door and click Go to continue the calibration.*

*After the dye plate has been scanned, the acquired data can be viewed and checked for anomalous readings. The average signal in at least one of the channels should be significantly greater than the blank plate signals for that channel (approx. 10X).*



*If results are satisfactory, click Next to proceed to the final screen. Add comments (if desired) to the calibration (e.g., record the date). Click Finish to save and complete the calibration process.*

*Repeat the calibration process for each dye and plate type.*

## 5) After calibration, back up your entire Opticon Monitor 3 directory.

*If you're in this section of the Guide, you've already lost all of your calibration data at least once. Don't let it happen again. Practice good habits by routinely backing up your critical software directories.*

## 6) Congratulations! You're finished!!!!

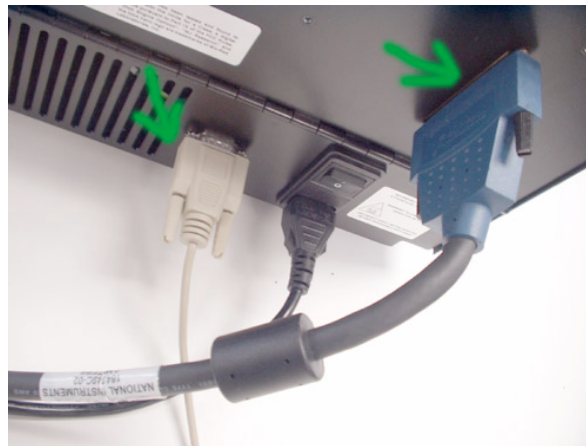
## Appendix C: Testing the computer serial port and cable.

- 1) You only need to test the computer serial port and cable if the Enumerator test indicates that the thermal cyclers are not responding.

*To test the computer's serial port and cable, we will use a "loopback" test. The serial cable has a transmit wire and a receive wire. If the two wires are connected together at the end of the cable, then any signals sent out from the computer will be returned back to the computer. The Enumerator software can easily identify whether this signal flow is working properly.*

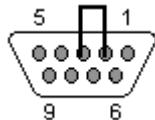
- 2) Disconnect the null-modem serial cable from the Opticon.

*The serial cable is the small beige cable that plugs into the lower part of the Opticon, Leave the other end of the cable connected to the serial port on the computer.*



- 3) Short out the transmit and receive pins on the end of the cable.

*The serial cable is the small beige cable that plugs into the lower part of the Opticon, Bend a paperclip such that the bare metal ends of the paperclip can be inserted into the adjacent holes of the transmit and receive pins on the end of the DB-9 cable connector (pins 2 and 3):*



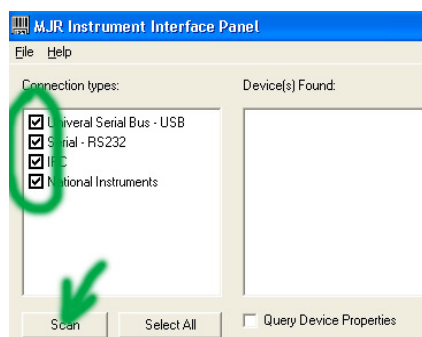
**Warning!** Do not insert the paper clip into any other holes in the end of the DB-9 connector! You can potentially damage the computer COM port by shorting the wrong holes.



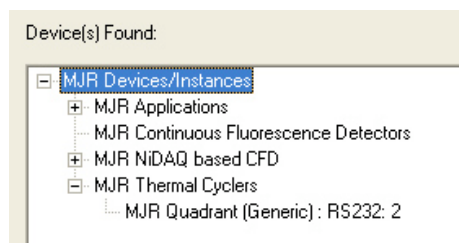
**Warning!** Do not insert the paper clip into any other cables. The serial cable contains only low voltage and is safe to contact with bare hands, but other cables may contain high voltage and should not be touched with a paper clip!

#### 4) Use the Enumerator software to determine if the serial communications are okay.

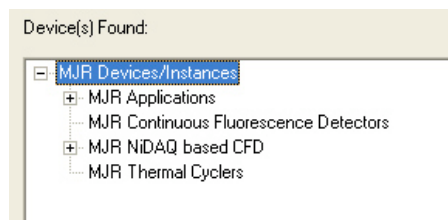
The Enumerator is a program called *IIEnum3.exe* in the *\opticon monitor 3\utils\* directory. Open the program, select all **Connection types**, and click **Scan**:



Look for the presence of the words **MJR Quadrant (Generic): RS232: 2** in the devices found list. Confirm that the quadrant was found on COM port number 2. (The COM port number is the digit that appears after RS232: in the identifier string).



Now remove the paperclip from the end of the cable. Click **Scan** again, and confirm that the instance of **MJR Quadrant (Generic): RS232: 2** is no longer on the list.



The above results indicate that the computer serial port and the null-modem serial cable are both working normally.

- **If the MJR Quadrant (Generic) is not found** when the cable is shorted by the paperclip, reposition the paperclip and try again. If it is still not found, then you either have a bad cable or a bad serial port. Try a different cable and then a different serial port.
- **If the MJR Quadrant (Generic) is found, but is not on COM2**, adjust your COM port settings so that the port number is correct and try again (Step 1 part 3 of the Guide).
- **If the serial port and cable are confirmed to be okay, but the Enumerator will not identify the PTC-200 when the cable is plugged directly into the Opticon**, then the failure of communications is with the DNA Engine inside of the Opticon. Check the following:

- *Is the serial cable properly plugged into the Opticon?*
  - *Is the Opticon powered on?*
  - *Confirm that the fuses in the power entry module outside of the Opticon are okay.*
  - *Confirm that the fuses in the power entry module of the PTC-200 inside the Opticon are okay (access is via door on rear of Opticon).*
  - *Confirm that the serial cable pigtail that connects the Opticon serial port with the PTC-200 serial port is properly plugged in (access via door on rear of Opticon).*
- ***If the serial port and cable are good and the PTC-200 is powered on, yet the Enumerator still will not identify the PTC-200, then there is a failure of communications within the PTC-200 itself. Field Service is required.***

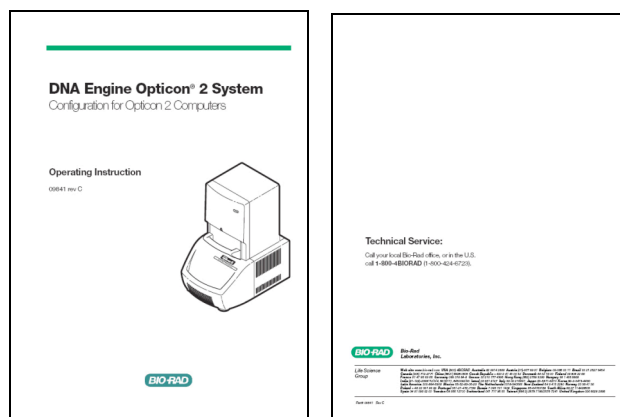
## Appendix D: General communications troubleshooting.

**1) This quick guide outlines the steps to check if the Opticon does not appear to be communicating with the computer. It is only applicable to Opticons running Opticon Monitor 3.1 software.**

- ✓ Verify that the Opticon is turned on and all cables are properly connected.
- ✓ Confirm that the computer is running Windows XP operating system.
- ✓ Confirm that the COM port on the computer is set to COM 2 (Step 1 part 3 of Guide)
- ✓ Use the M&A Software to check the NIDAQ card (Step 2 part 3 of Guide).
- ✓ Use the Enumerator to check the serial communications (Step 5 part 1 of Guide, or Appendix C).
- ✓ Verify proper firmware version on the cyclor (Step 5 part 1 of Guide).
- ✓ Confirm that the dye files have been properly installed (Step 6 of Guide, or Appendix A).
- ✓ Replace NIDAQ cable. Replace the null-modem serial cable.
- ✓ Call field service. In the USA, call 800-424-6723.

# Appendix E: New Instrument Installation.

1) If you are installing a new instrument, please follow the directions that came with the unit to avoid unnecessary steps. If you do not have these instructions, they are reprinted below.



## ■ Configuration for Opticon 2 Computers Operation Instruction

**1.1 PURPOSE:** This document outlines the steps required for proper configuration of computers to be used with an Opticon2 real-time instrument.

### 2.0 SCOPE:

2.1 This procedure applies to all Opticon2 computers being configured for operation using Opticon Monitor application.

### 3.0 RESPONSIBILITY:

3.1 The Manufacturing Engineering group in conjunction with QA department is responsible to ensure this procedure is accurate, understood and effectively implemented.

### 4.0 EQUIPMENT/OPERATOR MANUALS:

4.1 Refer to Opticon2 Operations Manual

### 5.0 DEFINITIONS:

5.1 N/A

### 6.0 PROCEDURE:

**Follow the steps to configure a computer for use with Bio-Rad Laboratories Opticon2 instruments "CFB32201G".**

6.1 In an Electro Static Discharge (ESD) - Safe location, remove the DAQ Board, Opticon Monitor Installation CD, and Opticon 2 Configuration CD from the shipping boxes.

6.2 Verify and record the serial numbers of the Opticon 2, DAQ Board and Computer and Monitor that will be matched to each other.

#### 6.3 NI-DAQ Installation

Insert the Opticon 2 Configuration CD into the CDRW drive. Open the NI-DAQ692 folder in the CD directory and launch the setup.exe file by double clicking on the icon. Follow instructions provided by the installation program. When prompted for the features to install, install only the components selected by default: NI-DAQ Device Driver and NI-DAQ OPC Server.

Restart the computer when prompted to do so.

Upon restart, the installer will prompt whether it should install documentation. Check the box to skip the installation of documentation: When given the option to either configure Measurement and Automation System or quit the setup, select the option to quit. Shutdown the computer

#### ■ Configuration for Opticon 2 Computers Operation Instruction

6.4 Using ESD-safe practices open up the case of the computer, remove the PCI slot cover and install the DAQ Board that was supplied with the Opticon (see exemplar picture below). Close the case.

6.5 Connect the 68-pin DAQ Cable between the Opticon 2 and the DAQ board connector.

Connect the 9-pin null modem cable between the Opticon 2 and the COM port on the rear of the Computer. The COM port number in the computer settings will need to be changed according to Section 6.8 below. If the computer has two COM ports, connect the 9-pin null-modem cable to the COM2 port.

Connect the Opticon 2 to an earth-grounded, surge protected electrical power strip rated for at least 15A at 120V or 10A at 200V.

Turn on the Computer, Opticon 2 and Monitor. When Windows loads, it will now recognize the new NI DAQ device and install it as a National Instruments device. Let Windows install the software for the National Instruments device automatically.

6.6 Once installation of the device driver is complete, open the Measurement & Automation program (Shortcut is located on the Windows desktop).

Press the F5 function key to refresh the configuration.

Click on the Devices & Interfaces icon to open the group, exposing the PCI-6036E DAQ device.

With the PCI-6036E entry highlighted, click on the Properties button.

Next click on the Test Resources button.

Confirm that a message "The device passes the test" is displayed. Then close Measurement and Automation.

6.7 Confirm the Power Options settings. Go to menu bar and select Start > Settings > Control Panel > Power Options. These should be the settings:

**System Standby: Never**

**Turn Off Hard Disks: Never**

**System Hibernate: Never**

6.8 If the computer had only one COM Port, the port number must be changed in the computer settings. Right-click on My Computer, select "Properties," then select "Hardware." Choose "Device Manager" and navigate to Ports (COM and LPT). Expand this section and double-click to select "Communications Port (COM1)." In the next window, select "Port Settings," click "Advanced" and change the COM Port Number to "COM2."

#### ■ Configuration for Opticon 2 Computers Operation Instruction

6.9 Install the Opticon Monitor software from the Opticon Monitor Installation CD provided in the Accessory Kit. When installing, do not change the default installation directory.

After installation completes, copy the instrument folder (OP00####) and its contents from the Opticon Configuration CD into the directory c:\Program Files\Opticon Monitor 3\Instrument\Opticon 2.

6.10 Launch Opticon Monitor software. Click to Accept the End User License Agreement.

6.11 Verify computer and NI DAQ functionality by performing the following:

Place a white plate (HSP-9655) into the Opticon.

Using Opticon Monitor software, open and run the Master file provided:

(Opt2\_TestProc.mast for the Opticon2)

Perform plate reads per master files provided for Opticon 2

(Opt2\_TestProc.mast).

After cycling and taking 5 plate reads, the program will automatically

stop

Review plate read data. The white plate data (Protocol step 6) should yield flat signals in all 96 wells.

Remove plate (HSP-9655) from instrument.

Exit Opticon Monitor software.

#### 7.0 MAINTENANCE/SAFETY:

7.1 N/A

#### 8.0 REFERENCE:

8.1 N/A

#### 9.0 APPENDIX:

9.1 N/A